# EXHIBIT D

**UNITED STATES DISTRICT COURT**

**SOUTHERN DISTRICT OF WEST VIRGINIA**

**CHARLESTON DIVISION**

| | |
|---|---|
| **IN RE: ETHICON, INC., PELVIC REPAIR SYSTEM PRODUCTS LIABILITY LITIGATION** | **Master File No. 2:12-MD-02327 MDL 2327**<br><br>**JOSEPH R. GOODWIN U.S. DISTRICT JUDGE** |
| **THIS DOCUMENT RELATES TO:**<br>***Janice Green v Ethicon, Inc., et al***<br>***Case No. 2:12-cv-02148*** | |

**Case Specific Report of Shelby F. Thames, Ph.D.**

**Janice Green v. Ethicon**

**June 17, 2016**

At my direction, Dr. Kevin Ong of Exponent Labs received tissue and mesh samples explanted from Janice Green herein labeled as Green 2.1 and 3.1. The Green 2.1 explant sample was implanted for some 2.1 years, and was received in a dry and thus non-preserved state. However, the Green 3.1 explant was implanted for 8.1 years before removal and was received preserved in 10% neutral buffered formalin. Dr. Ong divided the samples, rinsed and soaked them in distilled water, dried again and sent them to me via overnight delivery before any tissue removal or cleaning steps were undertaken. I received the 'before cleaning' samples labeled Green 2.1 and 3.1 along with an exemplar TVT mesh, (810041B, # Lot 3694576) an Ethicon product made of Prolene, the same material at issue in this litigation.

Upon receipt, the samples were examined via Light Microscopy (LM), Fourier Transform Infrared Microscopy (FTIR-Micro), and Scanning Electron Microscopy (SEM). The initial data acquired was designated as "Before Cleaning" and recorded. The explants were returned to Dr. Ong with my request to clean them according to the cleaning process I developed (see Figure 1). After cleaning through steps 1 and 2 of the aforementioned protocol, the "After Cleaning 1" explants were returned to me for further analyses. This

process was repeated as noted below in the cleaning protocol detailed in Figure 1. Explant examinations were conducted at the following intervals:

- Before Cleaning
- After Cleaning 1
- After Cleaning 2
- After Cleaning 3
- After Cleaning 4
- After Cleaning 5

| Sample Name | 1st Step | 2nd Step | 3rd Step | 4th Step | 5th Step | 6th Step |
|---|---|---|---|---|---|---|
| Green #2.1 Green #3.1 | Distilled water soak 1h | Desiccation drying 1 h, Analysis<br>Before Cleaning | Distilled water. Water bath (80°C), 20h | NaOCl. Shaker, 30min (#2.1) 10min (#3.1) | Distilled water. Rinse; soak 1h; Rinse | Desiccation drying 1 h, Analysis<br>After Cleaning 1 |

| Sample Name | 7th Step | 8th Step | 9th Step | 10th Step | 11th Step | 12th Step | 13th Step | 14th Step |
|---|---|---|---|---|---|---|---|---|
| Green #2.1 Green #3.1 | Distilled water. Water bath (80°C), 20h | NaOCl. Ultrasonic bath, 1.5h | Distilled water. Rinse, ultrasonic bath 1h, rinse. | Desiccation drying 1 h, Analysis<br>After Cleaning 2 | Distilled water. Water bath (80°C), 20h | NaOCl. Ultrasonic bath, 4h | Distilled water. Rinse, ultrasonic bath 1h, rinse. | Desiccation drying 1 h, Analysis<br>After Cleaning 3 |

| Sample Name | 15th Step | 16th Step | 17th Step | 18th Step | 19th Step | 20th Step | 21st Step | 22nd Step | 23rd Step |
|---|---|---|---|---|---|---|---|---|---|
| Green #2.1 Green #3.1 | Distilled water. Water bath (80°C), 20h | 0.8 mg/ml Proteinase K. Water bath (58°C), 20h | 0.8 mg/ml Proteinase K. Ultrasonic bath, 2h | Distilled water. Rinse, ultrasonic bath 1h, rinse. | Desiccation drying 1 h, Analysis<br>After Cleaning 4 | Distilled water. Water bath (80°C), 20h | NaOCl. Ultrasonic bath, 4h. | Distilled water. Rinse, ultrasonic bath 1h, rinse. | Desiccation drying 1 h, Analysis<br>After Cleaning 5 |

**Figure 1. Green 2.1 – Cleaning Protocol**

## Before Cleaning

The "Before Cleaning" samples were examined via light microscopy (LM), scanning electron microscopy (SEM), and Fourier transform infrared microscopy (FTIR-Micro). Figure 2 illustrates the appearance of a pristine TVT mesh (810041B, # Lot 3694576) sample. Light microscopy analysis (Figure 3, 4, and 5) depicts the extent to which the explanted meshes were tissue encapsulated.

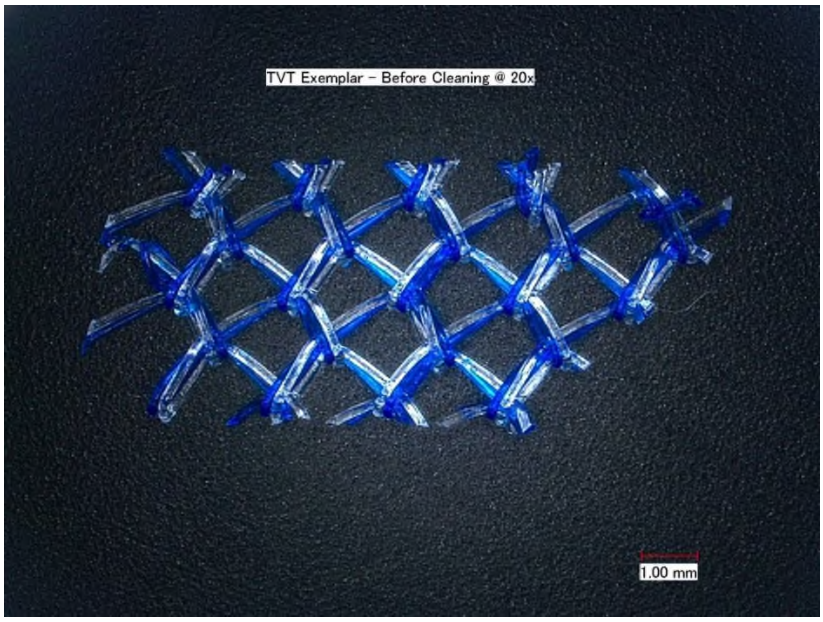


**Figure 2. Pristine TVT mesh (810041B, # Lot 3694576) – Before Cleaning**


**Figure 3. Green 2.1 sample (received dry) – Before Cleaning**

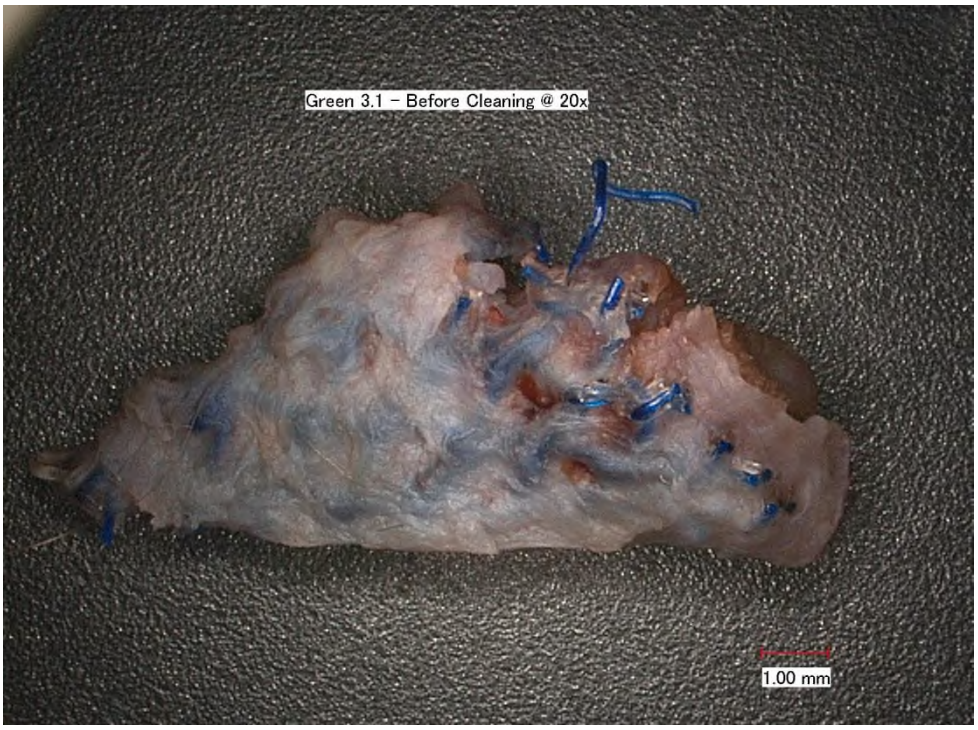


**Figure 4. Green 3.1 sample (received in Formalin) – Before Cleaning**

Higher magnification (100 – 200X) LM of the Prolene explants are shown encased within a dry and cracked, proteinaceous mass Before Cleaning and After Cleaning 1. Explant 2.1 is shown in Figures 5 and 6 and Explant 3.1 is shown in Figures 7 and 8.

Figures 3, 5 and 6 describe the condition of the Green-2.1 explant; the explant was received in a dry state and therefore was not "fixed" in a Formalin solution. Consequently, in the absence of formaldehyde, there was no opportunity for formalin-fixation to occur and for the development of a protein composite to adhere to and form around Prolene fibers. It is for this, and other reasons, that "non-fixed explants" such as Green 2.1 are not as difficult to clean as are fixed explants. For instance, non-fixed explants are covered by flesh and proteins, both of which are prone to decomposition if not otherwise protected such as with Formalin fixation. The non-protected flesh and its components decompose over time into a variety of fats, oils and other organic residues.[2add other reference also] Thus, in the case of the Green explants we find the two extremes for explant maintenance; one with no preservatives (Green 2.1) and the other (Green 3.1), preserved by the Formalin fixation process. The analytical data obtained for the two explants are much different as will be described below.

**Consider the Green 3.1 Explant:**

The LM data alone (Figures 7 – 8) defeats plaintiff's consistent and incorrect tenet of *in-vivo* Prolene degradation. Plaintiffs contend, without any scientific evidence, Prolene degrades *in vivo* with concomitant cracking, loss of physical integrity and toughness, loss of molecular weight, embrittlement, and so forth. Plaintiffs allege Prolene

undergoes surface cracking which leads to these property losses. However, if one simply examines Figures 7 and 8 it is obvious that surface cracking and peeling occurs on both Prolene clear (unpigmented) and blue (pigmented) fibers. Plaintiffs contend the surface cracking material is degraded Prolene. However, this cannot be true. For instance, if the surface cracking and peeling material is degraded Prolene, likewise the pigmented (blue) and supposedly degraded surface and cracking material would be blue. In the same way, the clear and unpigmented degraded Prolene clear fibers would be clear. That is not the case. The LM data shows unequivocally the material peeling on both the clear and blue Prolene fiber is translucent under light microscopy, and proves therefore that Prolene does not degrade/oxidize *in vivo*. Simply put, if the blue Prolene fiber degraded its degradation products would be blue, and no such material exists. What does exist, however, are translucent flakes of "formalin-fixed" proteins that form around the Green 3.1 explant (see Figure 8).

**Consider the Green 2.1 Explant:**

While both explants Green 2.1 and 3.1 are manufactured from Prolene, their explanting history is quite different. After surgical removal, Green 2.1 was maintained without preservatives. As a result flesh decomposition products formed around the Prolene fibers, as we have verified by FTIR spectroscopy. Moreover, since there was no opportunity for formalin-fixation to occur, the composite, formaldehyde-protein flakes, did not form and, as expected, are not seen by LM, SEM or FTIR. Furthermore, the flesh decomposition products found with Green 2.1 are not present with the Green 3.1 explant.

These findings clearly do not support plaintiff's theory that the cracked material is degraded Prolene. Instead, the LM data are proof positive that the cracked and peeling product is not Prolene. Figure 8 is an excellent example describing the presence and appearance of formalin-protein flakes covering both Prolene blue and clear fibers. FTIR has identified the translucent flakes as protein derived in composition, and not oxidized or degraded Prolene. These data are consistent with formation of a protein-formaldehyde composite coating around all Prolene fibers, blue and clear.


**Figure 5. Green 2.1 – Before Cleaning @ 200X**

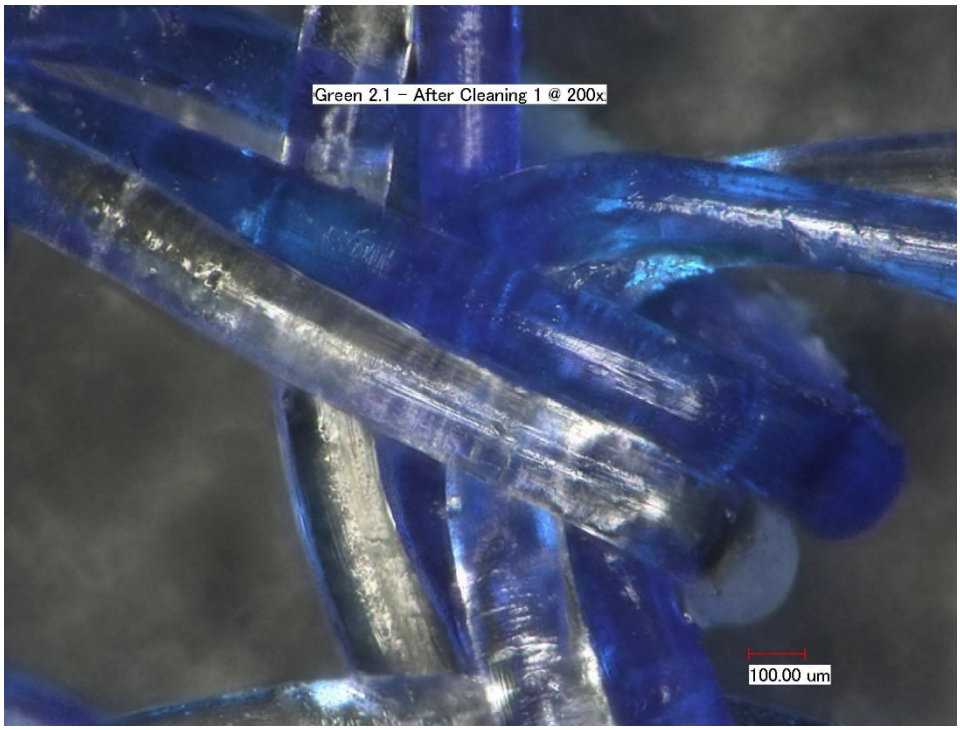


**Figure 6. Green 2.1 – After Cleaning 1 @ 200X**

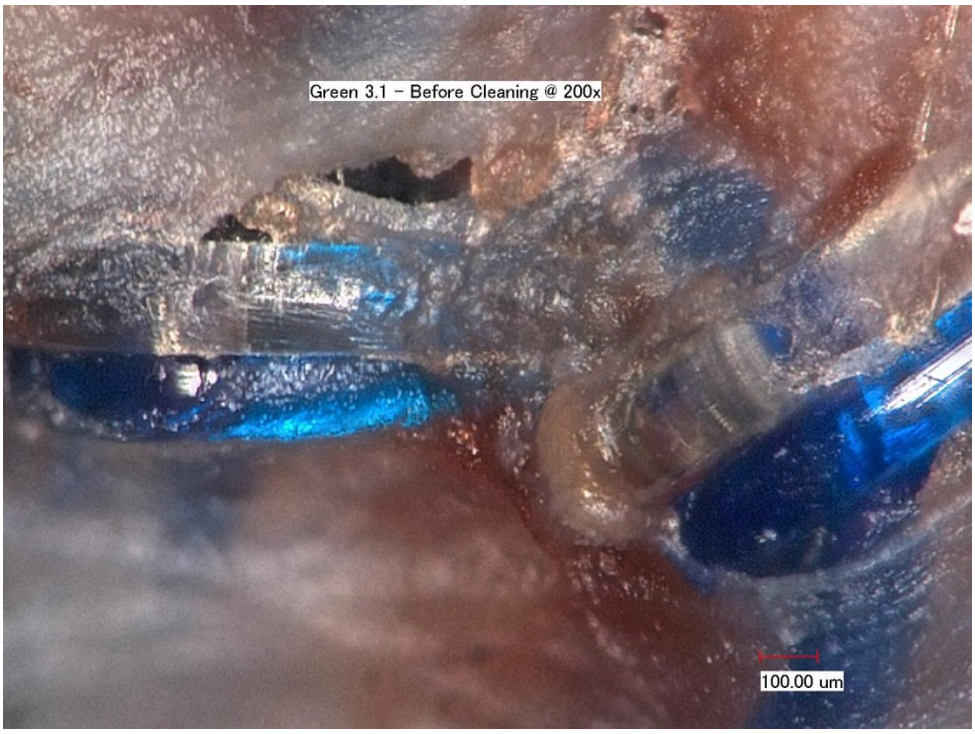


**Figure 7. Green 3.1 – Before Cleaning @ 200X**


**Figure 8. Green 3.1 – After Cleaning 1 @ 200X**

**Chemical Structure Analysis by FTIR Spectroscopy**

It is important to once again call attention to the translucent/clear appearance of the cracked and peeling material of both the blue and clear fibers of Explant 3.1, with an *in vivo* residence of 8.1 years. The peeling layer of the blue fiber is clear, not blue, and thus provides absolute confirmation its composition is not Prolene (see Green 3.1 – Figure 8). Degraded blue Prolene fibers would be blue and this peeling material is not blue; therefore, it is not Prolene. We have demonstrated via FTIR spectroscopy that it is of Protein origin.

The presence of the thin, and remaining, translucent protein layer on the Prolene fiber after flesh has been mechanically removed proves strong protein adsorption, and a strong adhesive bond between the adsorbed proteins and Prolene.

The "Before Cleaning fiber" FTIR spectra (Figures 10 and 11) shows spectral components of both polypropylene and proteins as noted by the highlighted 3297, 1653, and 1540 $cm^{-1}$ frequencies. These absorption frequencies are attributed to the required protein amide N-H stretching in the 3300 $cm^{-1}$ region, the amide I carbonyl stretching in the region of 1600-1690 $cm^{-1}$ and the amide II stretching and bending in the region of 1489 – 1575 $cm^{-1}$ as noted by Kong *et al*., respectively and illustrated in Figure 9.[1] Similar absorptions were observed for the Green 3.1 sample (Figure 11).

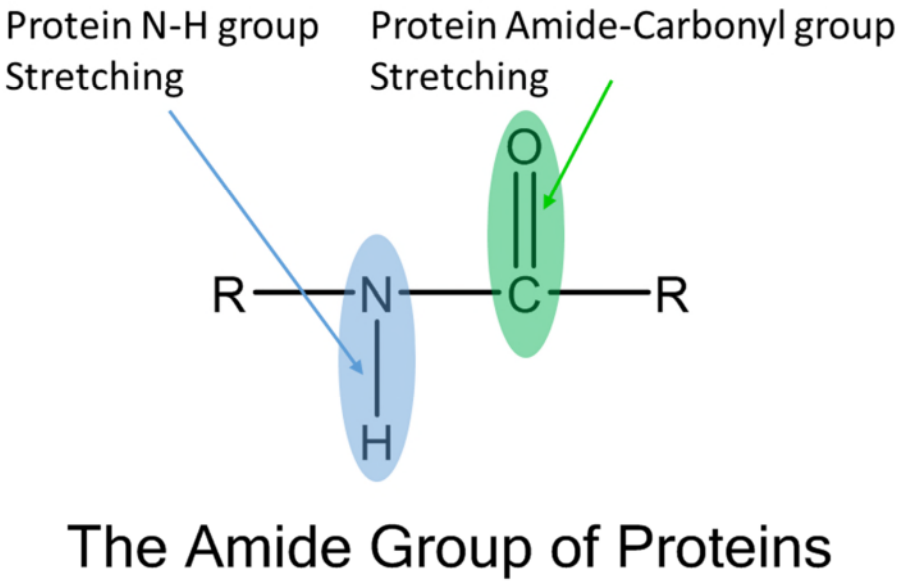


**Figure 9. The functional groups of amides**

Polypropylene absorption frequencies are also present at 1447 and 1381 $cm^{-1}$ due to penetration of the IR beam through both the protein layer and into the polypropylene fiber.

FTIR spectroscopy for explant Green 2.1 confirms the presence of tissue decomposition products comprised of fats and lipids surrounding Prolene fibers. It is known the process of tissue decomposition produces a number of fatty acids and esters as reported by Notter, Stuart, and Baker.[2,3,4] Similar decomposition products have been observed in other explants not stored in formalin, and thus not protected from decomposition. The

Green 3.1 explant, received and stored in Formalin, does not show decomposition products.

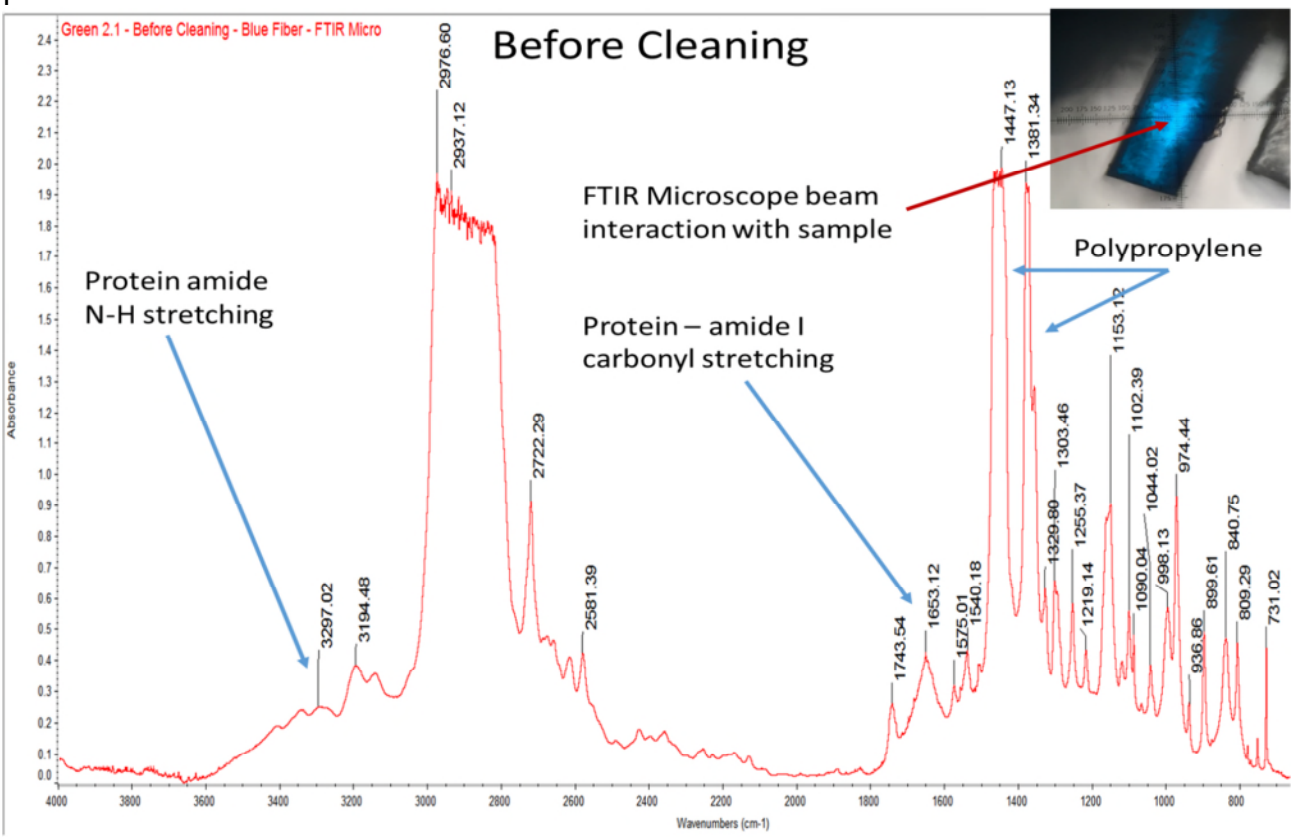


**Figure 10. Green 2.1 – Blue fiber FTIR before cleaning**

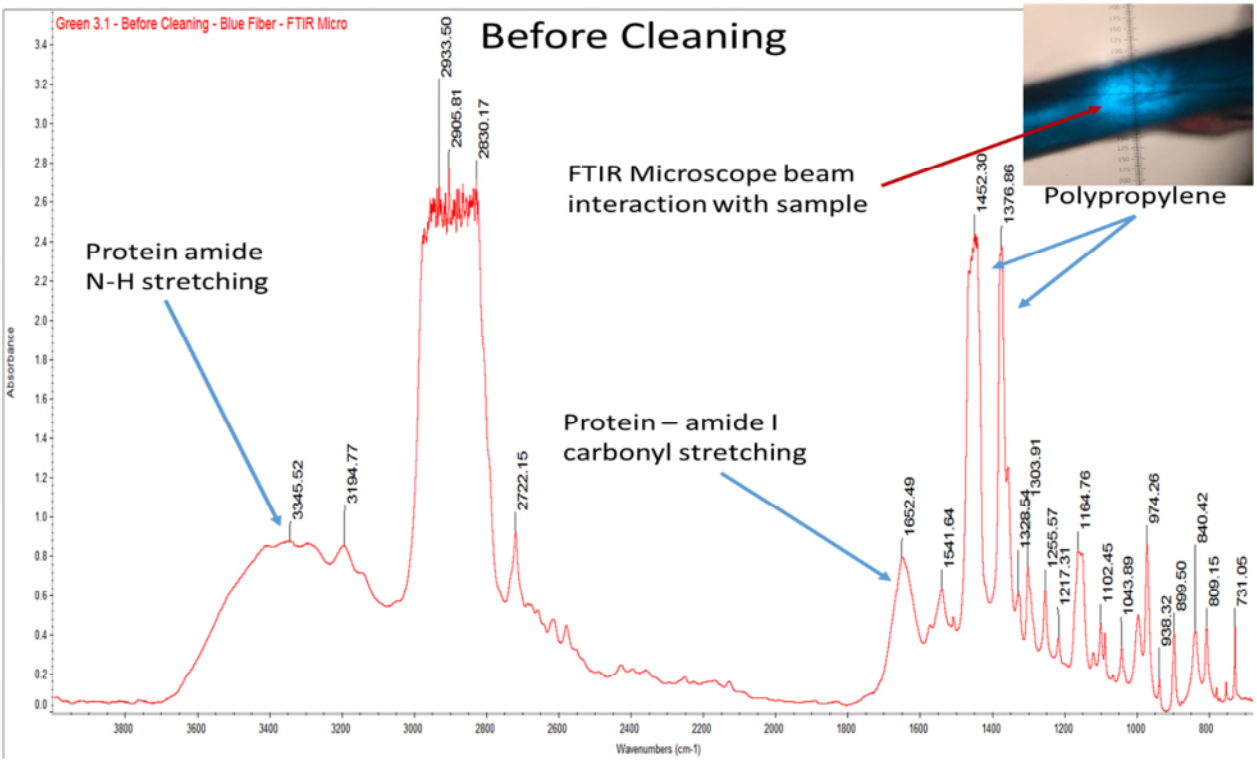


**Figure 11. Green 3.1 – Blue fiber FTIR before cleaning**

An FTIR spectrum of tissue located between the explant fibers Before Cleaning was collected and compared to the spectrum of Collagenase (a protein control) and they are included in Figure 12 demonstrating overlapping of the N-H, amide I, amide II absorption frequencies. The tissue spectrum was in turn overlaid with the spectrum of a fiber before cleaning demonstrating the amide peaks are present in both tissue and on the surface of Prolene fiber(s) (Figure 13).

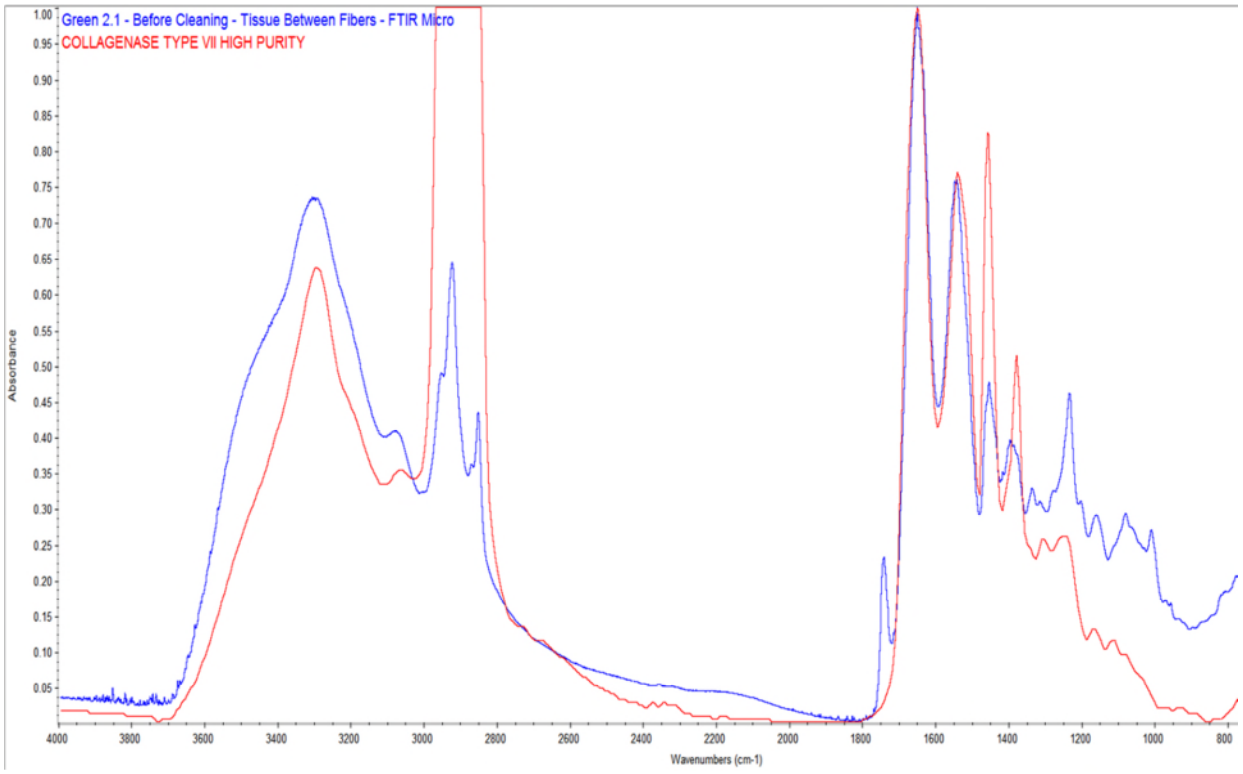


**Figure 12. Green 2.1 – Tissue between fibers Before Cleaning overlaid with a Collagenase reference spectra**

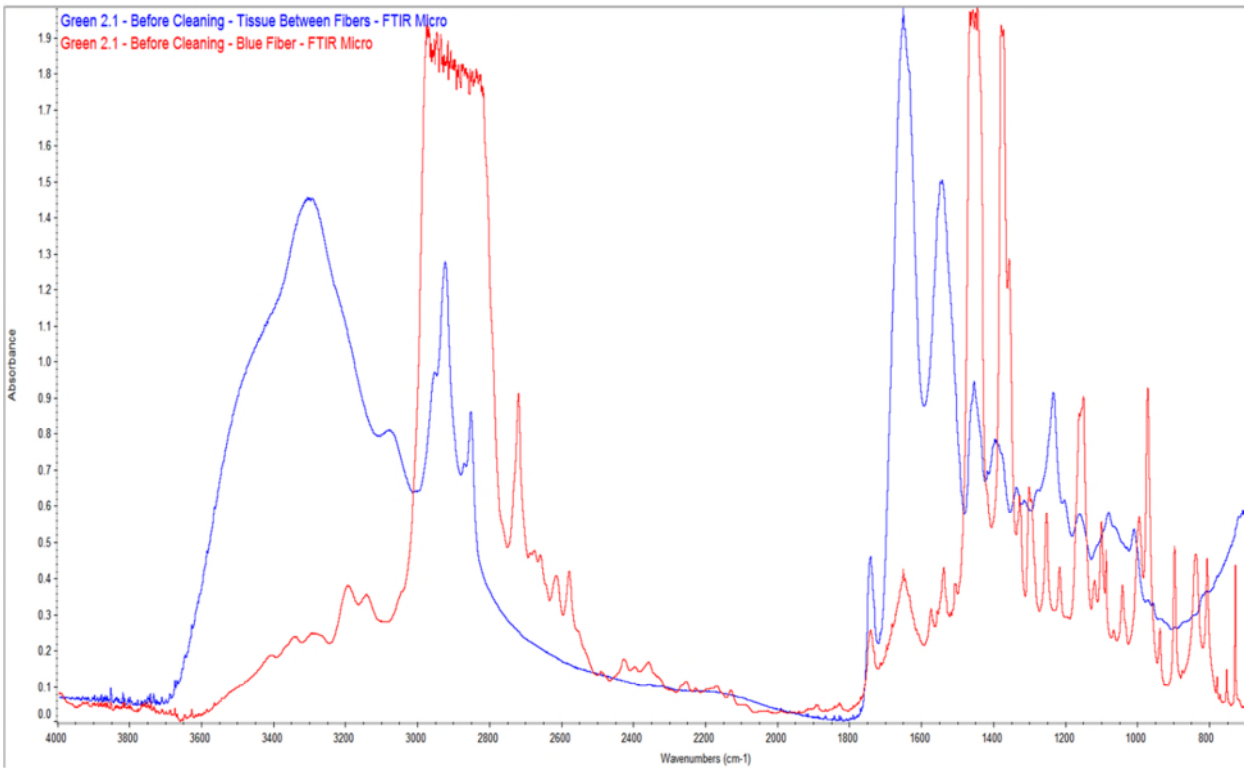


**Figure 13. Green 3.1 – FTIR of Tissue between fibers After Cleaning 1 overlaid with Blue Fiber before cleaning**

An Exemplar and explanted samples were likewise examined before and after the cleaning steps described (Figure 1). FTIR data demonstrates the progression of protein removal with cleaning of the explanted Prolene fibers (Figures 14 – 17). This was confirmed via light microscopy and SEM (see the series of images in Figures 21 – 26 after progressive explant cleaning).

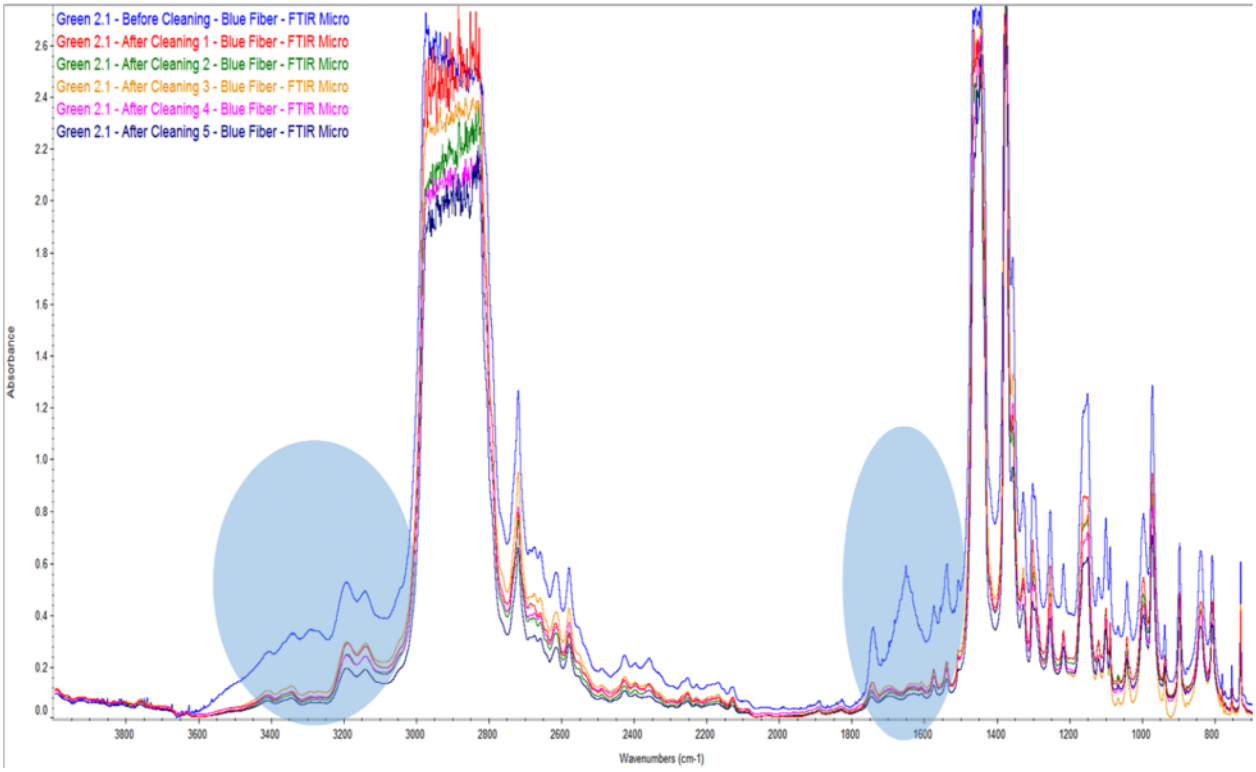


**Figure 14. Green 2.1 – FTIR of Blue Fiber after 5-cleaning steps**

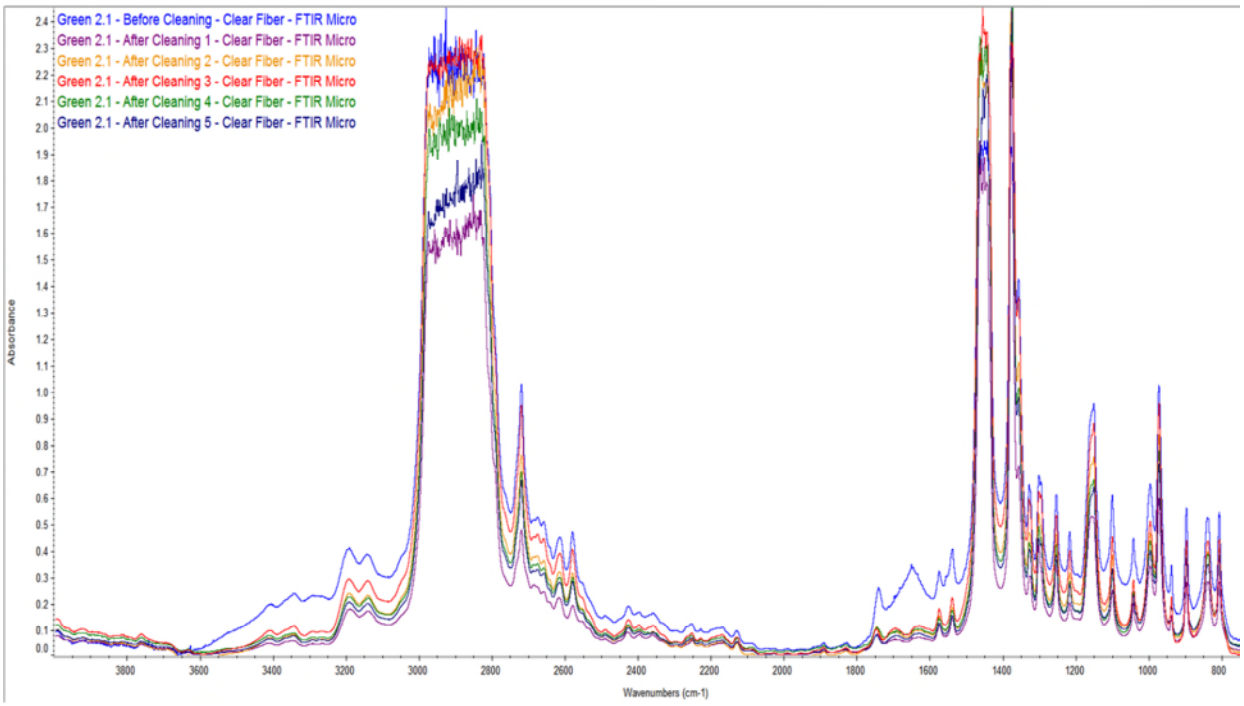


**Figure 15. Green 2.1 – FTIR of Clear Fiber after 5-cleaning steps**

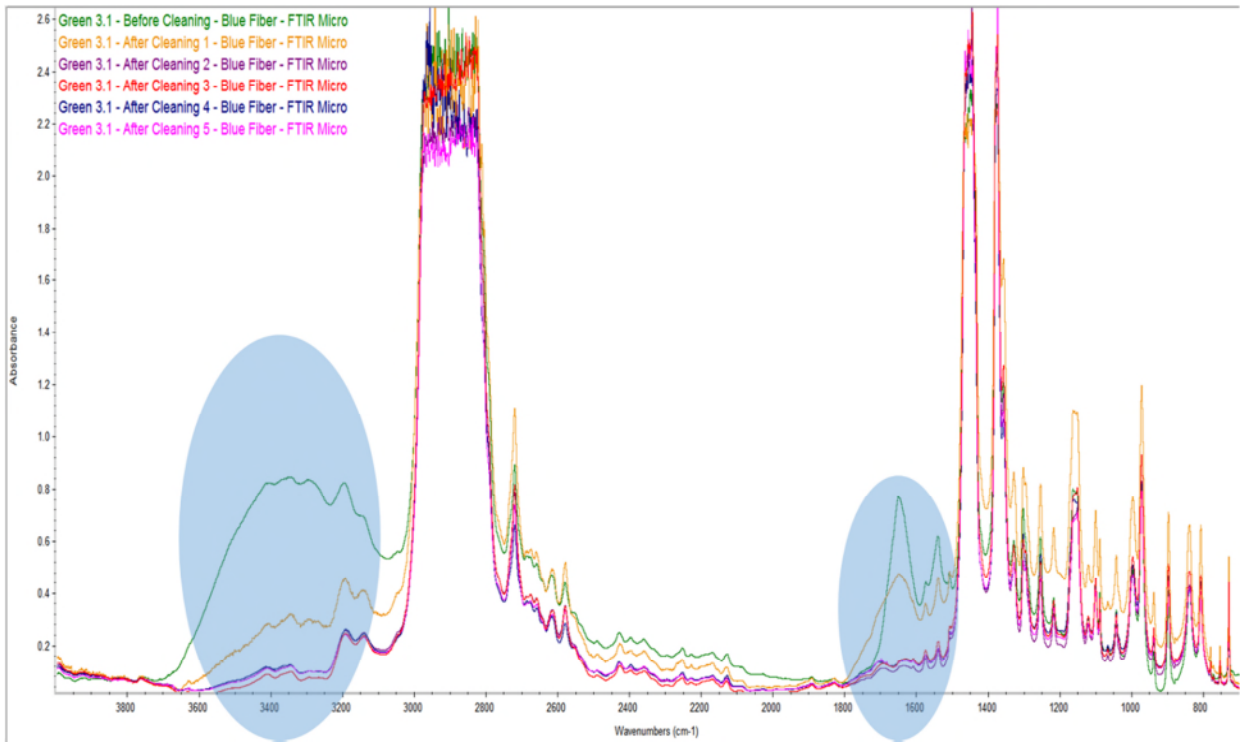


**Figure 16. Green 3.1 – FTIR of Blue Fiber after 5 cleaning steps**

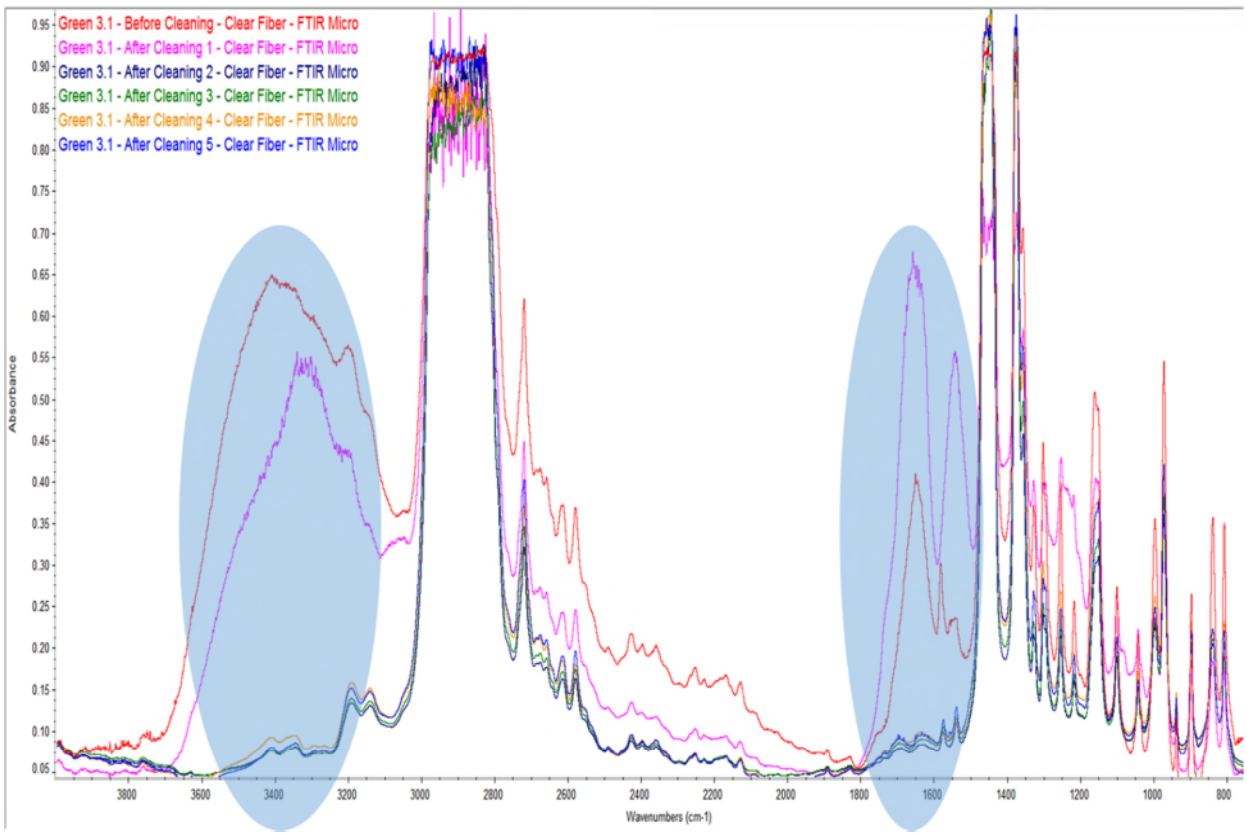


**Figure 17. Green 3.1 – FTIR of Clear Fiber after 5 cleaning steps**

The FTIR data proves unequivocally the surfaces of uncleaned explants are covered with adsorbed proteins. Furthermore, it has been known for more than sixty years that Collagen and other proteins chemically react with formalin during the explant-fixation process and in so doing form a tightly adhered, hard, brittle, insoluble composite polymeric sheath around the explanted fibers.[5] Consequently, in order to evaluate the structural and chemical status of the Prolene explant, the formalin-fixed protein layer must be removed. It is also important that during the formalin-fixed protein removal the Prolene explant is unaltered by cleaning reagents/process.

The chemistry controlling the formalin-protein fixation process is ideal for achieving a non-destructive cleaning protocol. For instance, the mechanism of this reaction is known to be reversible,[6] and its reversibility is the chemical basis for the cleaning protocol I developed (Figure 1). This cleaning process simply utilizes the reversible reaction by adding excess water to the “formalin fixed fiber” and heating at an elevated temperature (Figure 18).

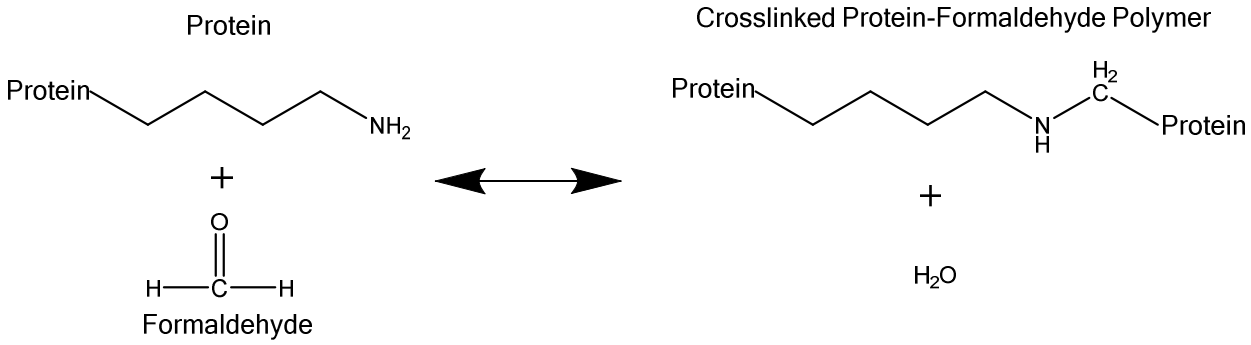


**Figure 18. Formaldehyde reactions with proteins during the fixation process.**

The efficacy of this cleaning process has been affirmed by FTIR, LM, and SEM microscopy as noted in the LM and SEM images of Figures 21 – 26. An example of this highly effective cleaning process is shown in the FTIR spectral overlays of Figures 19 – 20, comparing a TVT exemplar to the cleaned Green explants. It should be noted that the overlays confirms essentially identical structures for the cleaned explants and Prolene exemplar. FTIR data confirms a complete absence of any suggestion that Prolene was oxidized/degraded *in vivo.*

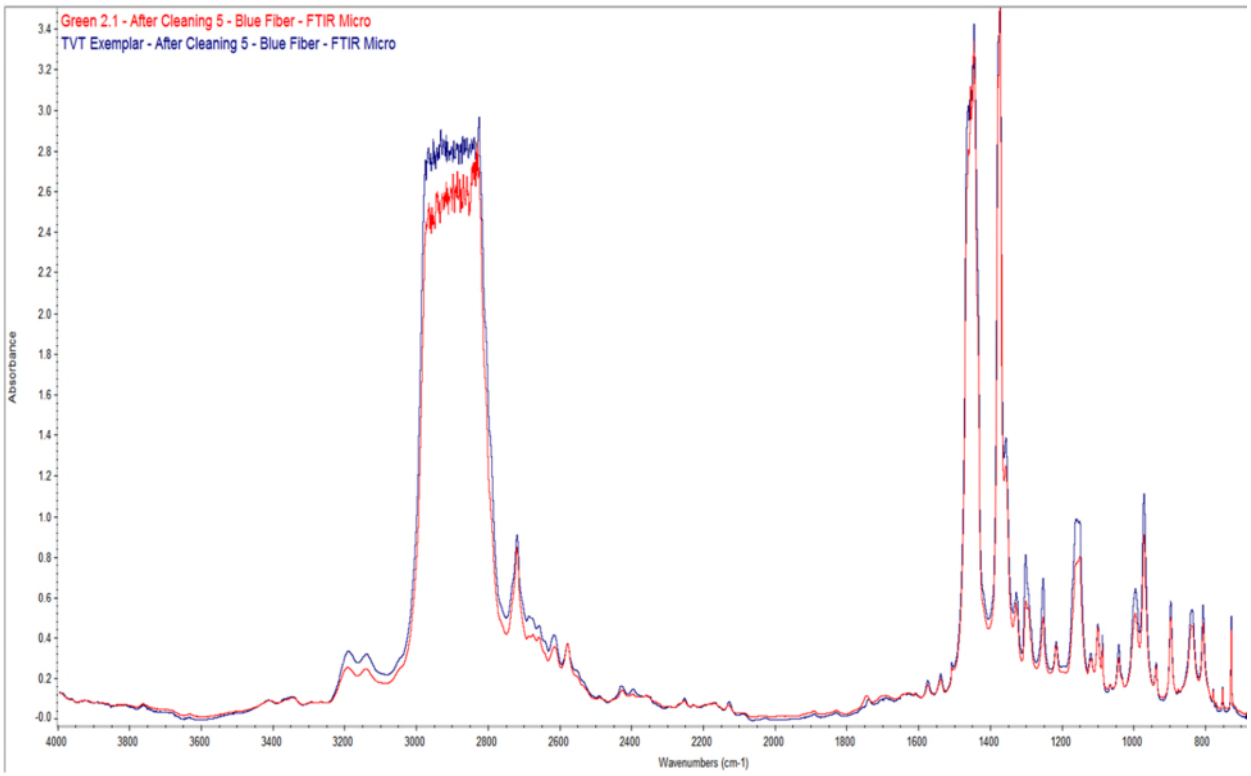


**Figure 19. Green 2.1 and TVT Exemplar – FTIR of Exemplar and Blue Fibers after cleaning steps**

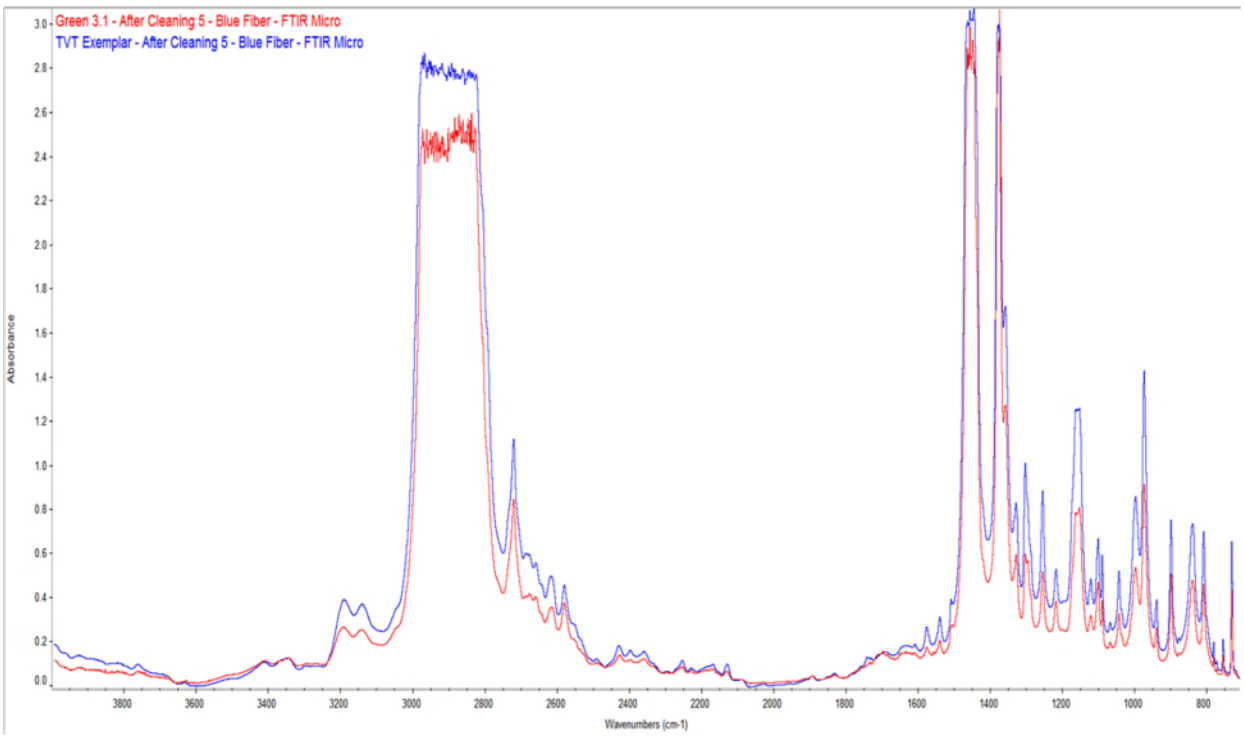


**Figure 20. Green 3.1 and TVT Exemplar – FTIR of Exemplar and Blue Fibers after cleaning steps**

The light microscopy images of Figures 21 and 22 illustrate the overall appearance of the explants before cleaning, and after each of the cleaning intervals previously discussed. Higher magnification light microscopy images are also included in Figures 23 and 24 demonstrating the successful removal of tissue during the cleaning protocol.

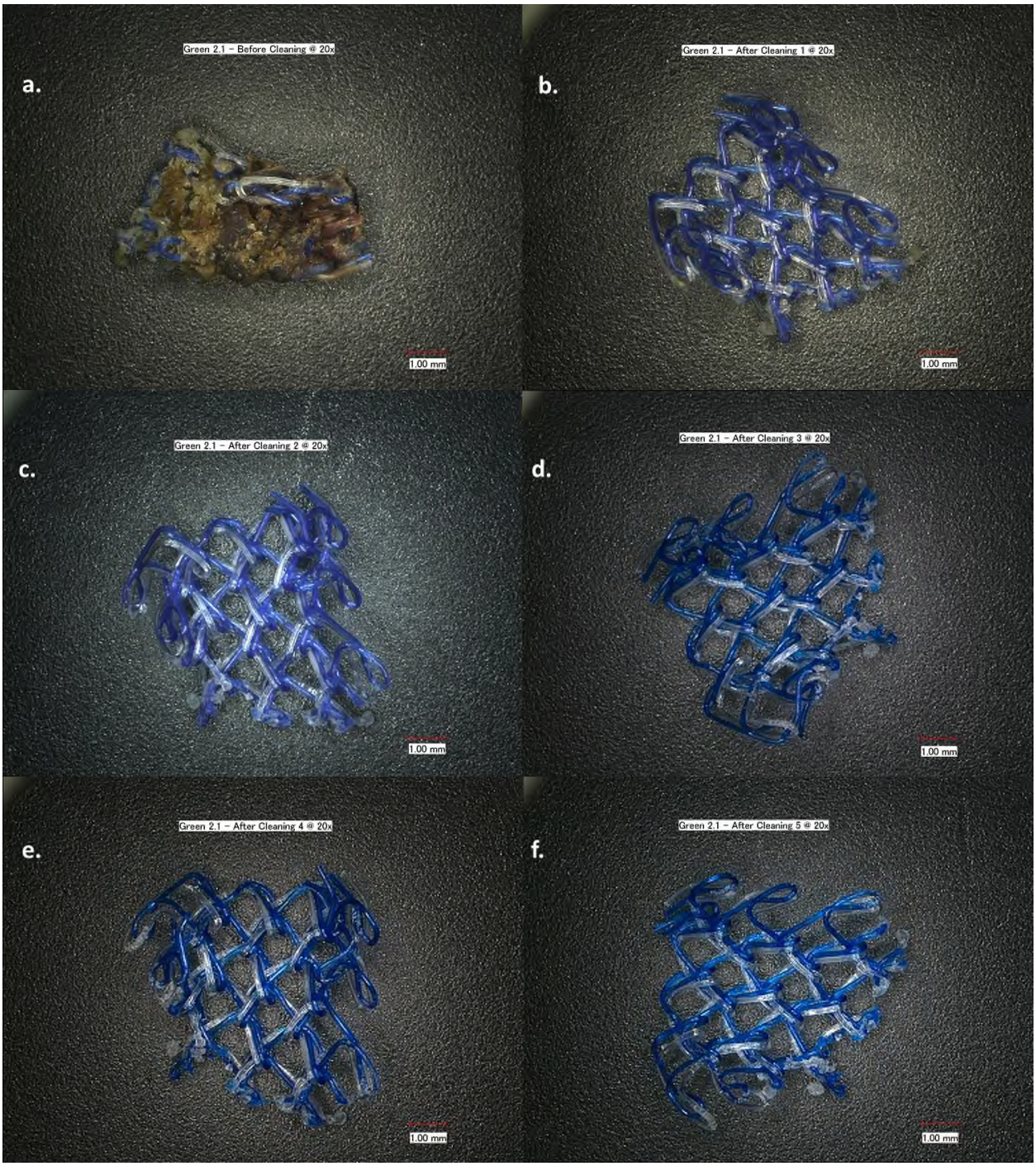


**Figure 21. a., b., c., d., e., and f. – Green 2.1 Light Microscopy after cleaning steps**

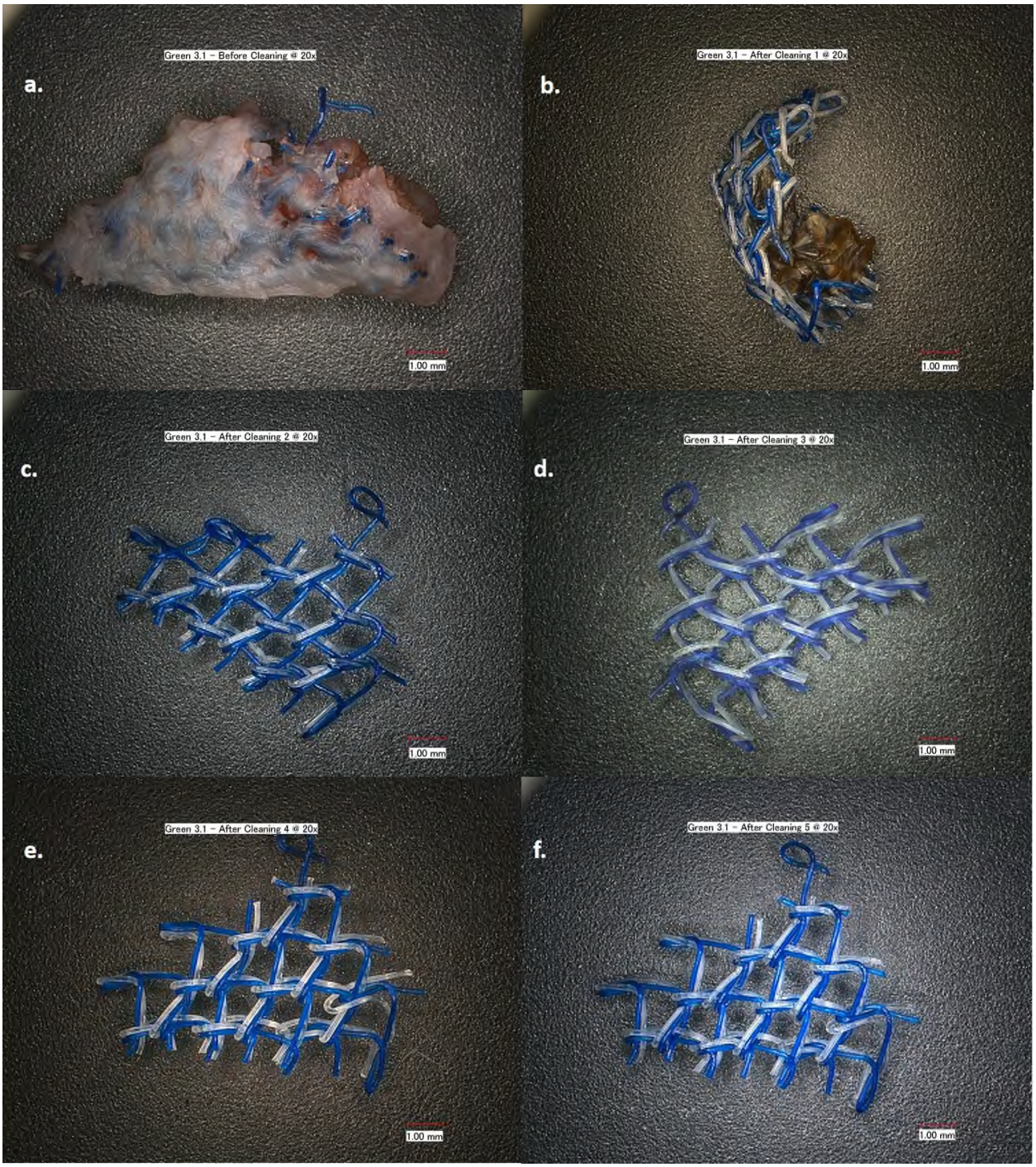


**Figure 22. a., b., c., d., e., and f. – Green 3.1 Light Microscopy after cleaning steps**

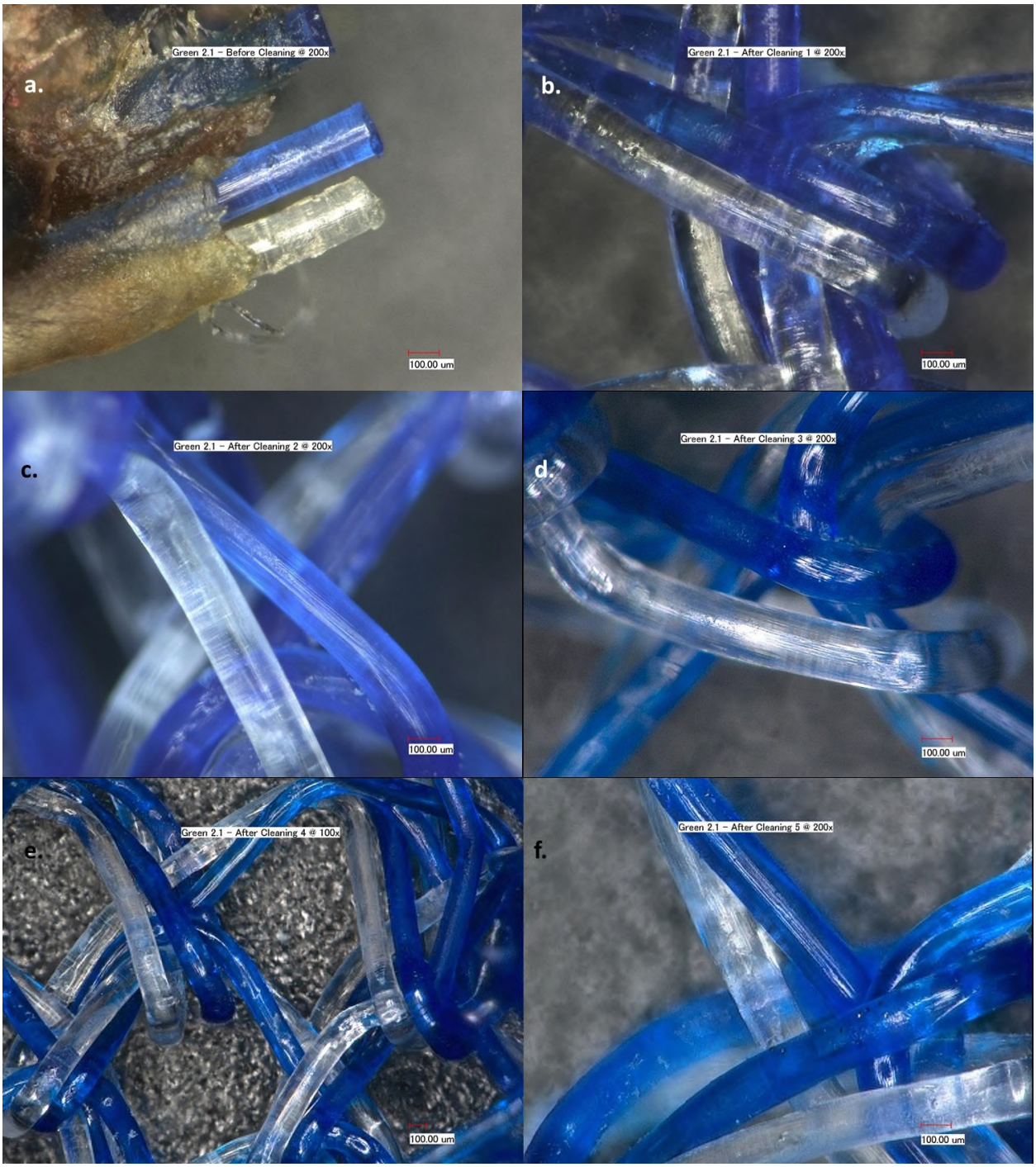


**Figure 23. a., b., c., d., e., and f. – Green 2.1 Light Microscopy after cleaning steps (100 – 200X)**

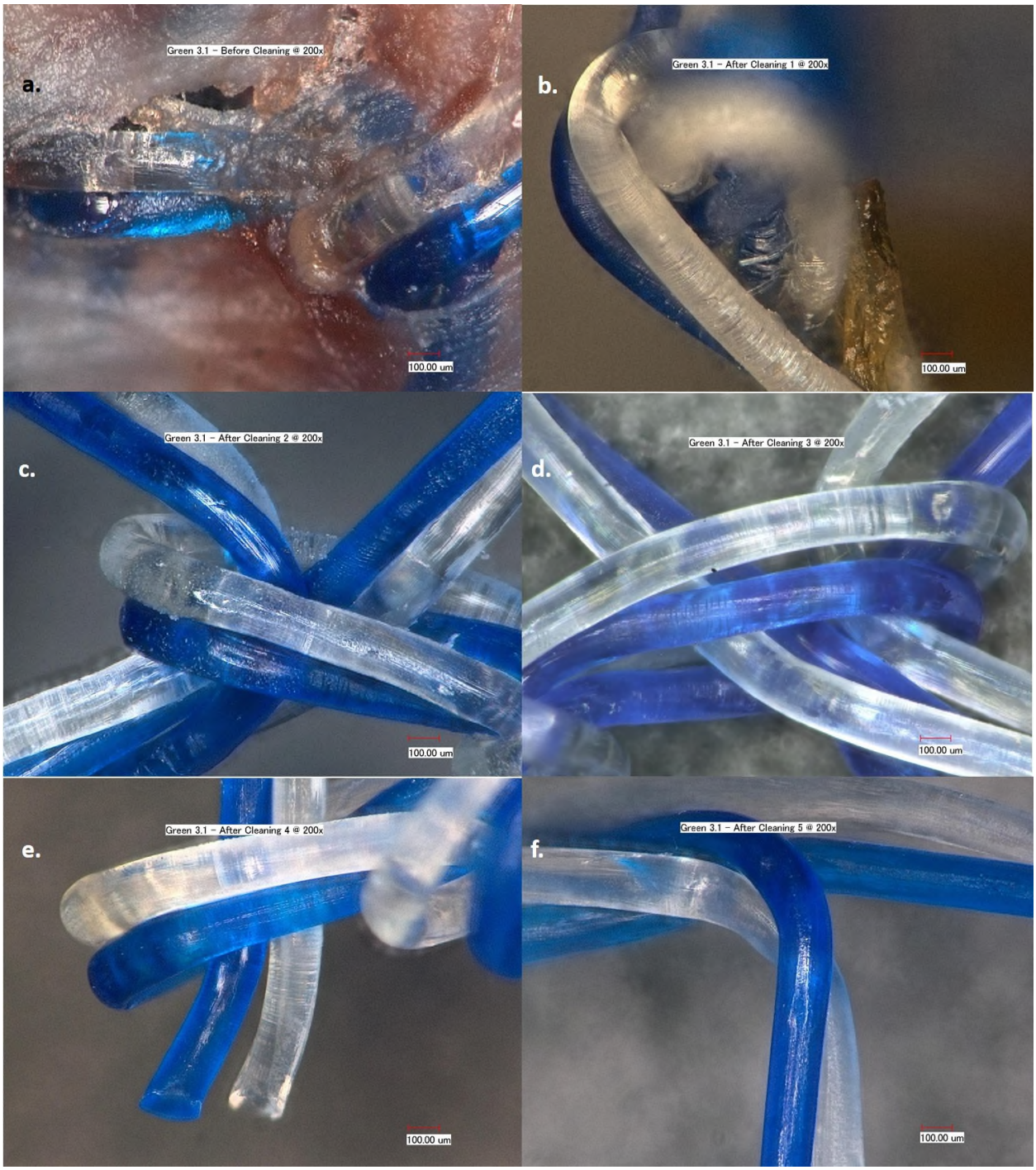


**Figure 24. a., b., c., d., e., and f. – Green 3.1 Light Microscopy after cleaning steps (200X)**

SEM images at various cleaning stages depicts both the progression of explant fiber cleaning as well as the tenacity with which proteins adheres to Prolene. Figures 25a and 26a are excellent examples of the surface appearance of fibers. The easily observable formalin-protein coating (Fig. 26) perfectly describes cohesive failure of the composite coating (see Figure 26b) and partial adhesive loss of this same formalin-protein composite layer from Prolene. Furthermore, the cleaning process demonstrates visually that surface degradation of Prolene did not occur. The cleaned fibers in Figures 25f and 26f "after cleaning 5" for example, continues to possess pristine-like extrusion lines created during manufacture. If the surface of the Prolene fibers degraded *in vivo*, as postulated by plaintiff's expert, the extrusion lines would likewise degrade during this process and would no longer be visible – that is not the case observed. There is no evidence of surface pitting or fracturing of Prolene in either Green 2.1 or 3.1. These data are additional evidence that surface oxidation/degradation of the Green Prolene implant did not occur *in vivo*. In conclusion, there is no data of which I am aware that even suggests oxidative degradation of Prolene occurs in vivo.

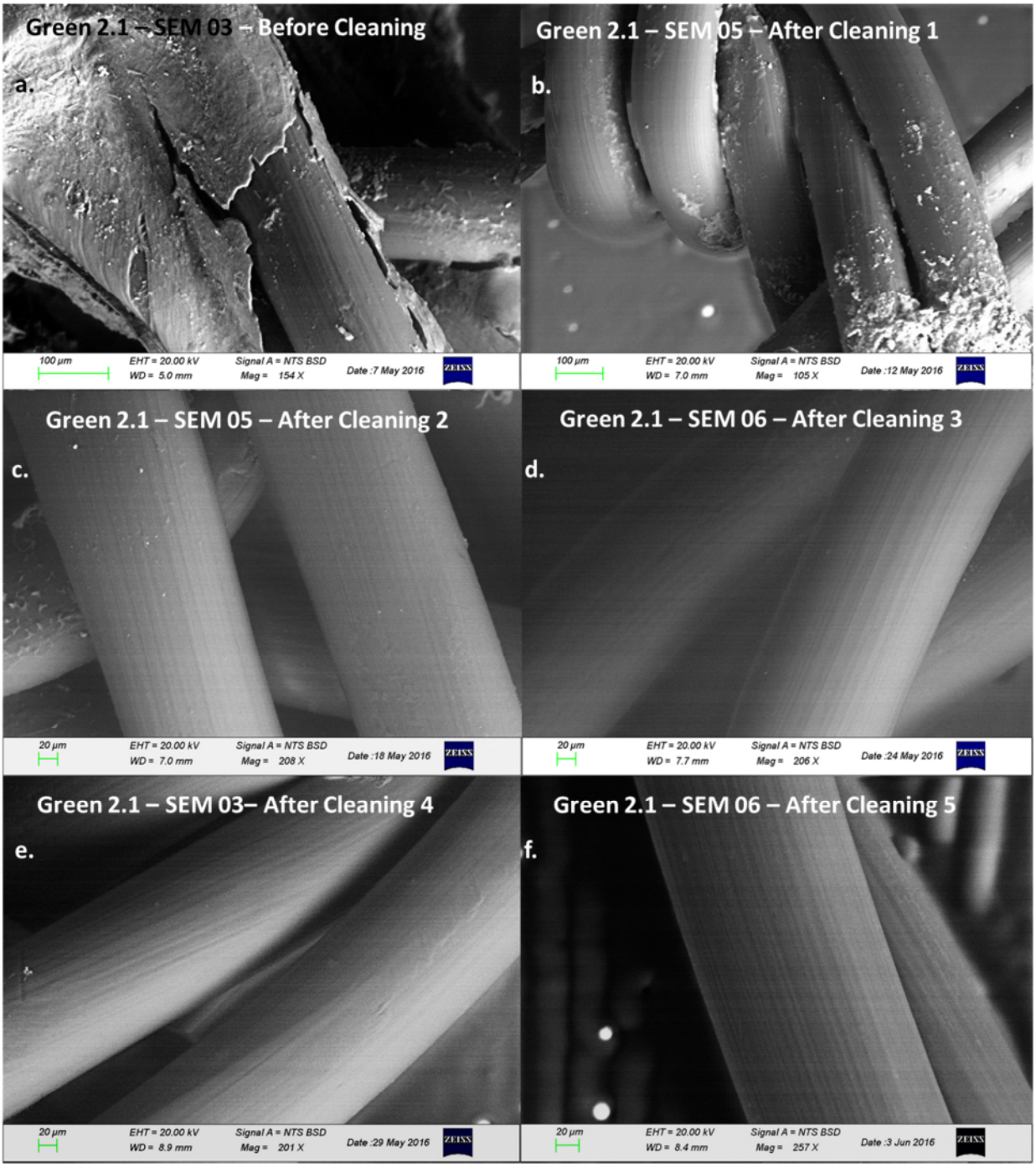


**Figure 25. a., b., c., d., e., and f. – Green 2.1 SEM Micrographs after cleaning steps**

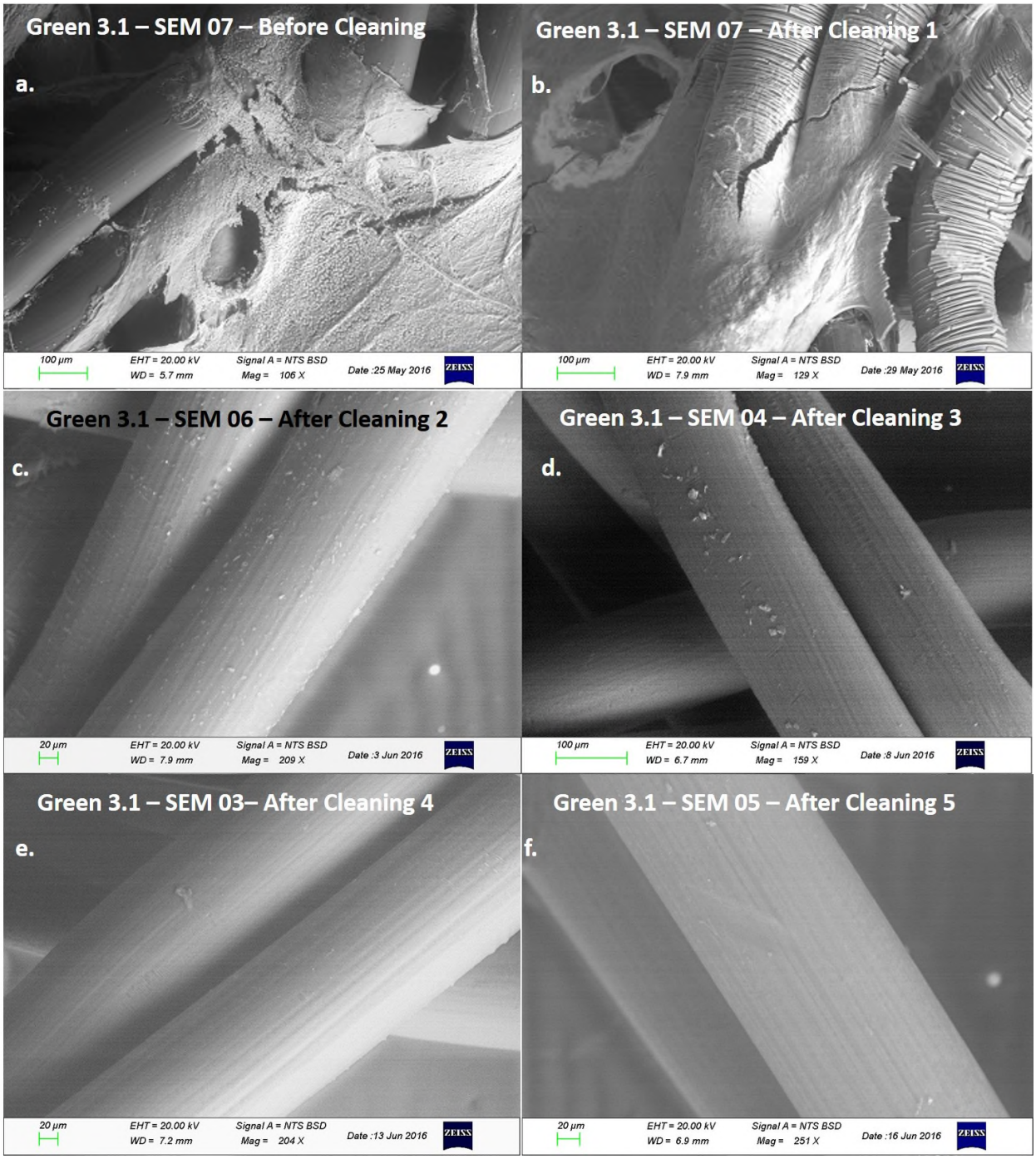


**Figure 26. a., b., c., d., e., and f. – Green 3.1 SEM Micrographs after cleaning steps**

I reserve the right to supplement this initial report and analysis, create additional exhibits as necessary to illustrate my testimony based upon the receipt of additional information, documents and materials, and to revise this report following the receipt of additional information and/or materials that have not yet been made available. Appendices of all FTIR, LM, and SEM data which I relied upon will be supplemented.

Shelby F. Thames, Ph.D.

[1] Kong, J. and Shaoning, Y., Fourier Transform Infrared Spectroscopic Analysis of Protein Secondary Structures, Acta Biochimica et Biophysica Sinica 2007, 39(8): 549-559

[2] Notter, S. J., Stuart, B. H., Rowe, R. and Langlois, N. (2009), The Initial Changes of Fat Deposits During the Decomposition of Human and Pig Remains. Journal of Forensic Sciences, 54: 195–201. doi:10.1111/j.1556-4029.2008.00911.x

[3] Stuart, Barbara, H. (2004) Infrared Spectroscopy: Fundamentals and Applications, Analytical Techniques in the Sciences, ISBN: 9780470854280, Wiley

[4] Baker MJ, Trevisan J, Bassan P, et al. Using Fourier transform IR spectroscopy to analyze biological materials. Nature protocols. 2014;9(8):1771-1791. doi:10.1038/nprot.2014.110

[5] Fraenkel-Conrat, Heinz. and Mecham, Dale, K., The Reactions of Formaldehyde with Proteins VII., Demonstration of Intermolecular Crosslinking by Means of Osmotic Pressure Measurements, J. Biol. Chem., 1949, 177:477-486

[6] Cordula Klockenbusch and Juergen Kast, “Optimization of Formaldehyde Cross-Linking for Protein Interaction Analysis of Non-Tagged Integrin 1,” Journal of Biomedicine and Biotechnology, vol. 2010, Article ID 927585, 13 pages, 2010. doi:10.1155/2010/927585